Original Article

Serum Versus Vitreous VEGF A and Central Macular Thickness in Diabetic Macular Edema and the Effect of Intra-Vitreal Bevacizumab on These Variables

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Pak J Ophthalmol 2016, Vol. 32 No. 2

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Received: March 31, 2016
Accepted: June 05, 2016

Purpose: To determine a relation among serum VEGF A, vitreous VEGF A and central macular thickness and the effect of intravitreal Bevacizumab on these variables.

Study Design: Quasi – experimental study.

Place and Duration of Study: Ghurki Trust Teaching Hospital, Lahore. 2014 to 2016.

Material and Methods: Fifty patients with clinically significant macular edema diagnosed with 78-D indirect Ophthalmoscopy were recruited. Optical coherence tomography (OCT for macular thickness), serum VEGF A and vitreous VEGF A were analyzed at pre and post (four weeks) intra-vitreal Bevacizumab injection. Measurement of Central sub field thickness, macular cube volume, cube average thickness, vitreous and serum VEGF A levels one month after a single injection of intra-vitreal Bevacizumab were the outcome variables.

Results: Out of fifty patients, forty three patients were included with an average age of 56 years. Mean pre-injection VEGF A values were 347.8 ± 92 pg/ml which were reduced to 107.2 ± 16 pg/ml four weeks post intra-vitreal Bevacizumab (*p* = 0.000). Mean pre-injection VEGF A level in vitreous was 701.9 ± 145 pg/ml and post injection 82.66 ± 21 pg/ml (*p* = 0.000). Pre and post injection central sub field thickness (CST) was 410 ± 24 µm and 299.6 ±15 µm respectively with *p* values of 0.000. Mean pre injection cube volume (CV) and cube average thickness (CAT) were 12 ± 0.36 mm^3 and 355.7 ± 10 µm which were reduced to 11± 0.26 mm^3 and 304 ± 9 µm after injection.

Conclusion: From this study, a direct relation of vitreous VEGF A, serum VEGF A and central sub field thickness is confirmed. Statistically significant absorption of intra-vitreal Bevacizumab occurs into the systemic circulation and effect of intra vitreal Bevacizumab on the fellow eyes is an additional benefit.

Key words: VEGF A, Diabetic macular edema, Bevacizumzb, Central Macular thickness, Cube average volume.

Diabetic macular edema (DME) is the commonest cause of decreased visual acuity in diabetic patients. The role of VEGF A in pathogenesis of diabetic macular edema is well documented[1]. VEGF A is released in response to hypoxia and has an important role in increasing the permeability of retinal vessels. This, ultimately results in macular edema[2,3,4]. Anti-VEGF agents are now widely used all over the world for treating DME. Many studies from Pakistan are also available which

show an improvement in diabetic macular edema after intra-vitreal injection of anti-VEGF agents.[5,6] Quantitative evidence of this improvement is possible with the help of OCT which can give macular thickness in micrometers.

In this study, we tried to find out the relation of serum and vitreous VEGF A levels with macular thickness. Effect of intra-vitreal Bevacizumab on serum and vitreous VEGF A and macular thickness of the treated and fellow eyes is also determined.

MATERIALS AND METHODS

An interventional, time series study was designed, in which fifty diabetic patients with clinically significant macular edema (CSME) were selected from Ghurki Trust Teaching Hospital, Lahore, Pakistan. The project was approved by Institutional Review Board.

Patients with type 2 diabetes having diabetic macular edema were included in the study. The following patients were excluded: patients with type 1 diabetes, patients with any other systemic disease, elevated blood pressure, and evidence of vitreoretinal interface abnormality on SD-OCT, and intra-vitreal corticosteroids or anti VEGF A agents or laser photocoagulation during last 6 months.

The primary outcome measures were a positive relation between high serum and vitreous VEGF A levels with central sub field thickness. Secondary outcomes were effects of intra-vitreal Bevacizumab on the central sub field thickness of treated and fellow untreated eyes.

Clinical history, including ocular as well as systemic history was taken. Ocular examination included distance and near visual acuity, pupillay reactions to light and accommodation, Slit-lamp Biomicroscopy and Tonometry. CSME was diagnosed with the help of +78D lens indirect Ophthalmoscopy. Macular thickness was measured using SD – OCT. (Carl Zeiss, USA, model 4000). Macular thickness parameters were; Central subfield thickness, Average cube thickness and Macular volume. On the very next day of OCT, blood and vitreous samples were drawn using strict aseptic techniques and Bevacizumab 1.25 mg in 0.05 ml was injected in the vitreous cavity. Patient was given topical antibiotics QID. OCT was repeated after four weeks and blood and vitreous samples were drawn again. Serum was separated from all blood samples and stored at -20°C. Patient proforma and consent forms were filled before sampling.

The methodology used in the current study to analyze VEGF A in vitreous and serum samples was xMAP flow cytometry and the kit used was Human angiogenesis/Growth factor magnetic bead panel. Flow cytometry is a simultaneous measurement of multiple physical characteristics of a single cell as the cell flows in suspension through a measuring device. It measures the optical and fluorescence characteristics of a single cell.

The statistical analysis was done by using SPSS version 21. Asymptotic Z test and Wilcoxon signed Rank tests were used for finding statistical significance.

RESULTS

Average age of the patients was 56 years (39-75 years). There were 17 (39.5%) males and 26 (60.5%) females. 18 right and 25 left eyes were studied. Mean pre-injection VEGF A values were 347.8 ± 92 pg/ml which were reduced to 107.2 ± 16 pg/ml four weeks after intra-vitreal Bevacizumab (p = 0.000). Mean pre-injection VEGF A level in vitreous was 701.9 ± 145 pg/ml and post injection 82.66 ± 21 pg/ml (p = 0.000). Pre and post injection central sub field thickness (CST) was 410 ± 24 µm and 299.6 ± 15 µm respectively with p values of 0.000. Mean pre injection cube volume (CV) and cube average thickness (CAT) were 12 ± 0.36 mm^3 and 355.7 ± 10 µm which were reduced to 11 ± 0.26 mm^3 and 304 ± 9 µm after injection. Mean CST, CV and CAT in the fellow non-treated eyes were 345.4 µm, 11.9 mm^3 and 329.2 µm respectively. These parameters were also significantly changed after injection of Bevacizumab in the other eye (CST = 282.8 µm, CV = 11.2 mm^3, CAT = 318.1 µm). For details refer to Tables 1, 2 and Figures 1, 2 and 3.

DISCUSSION

Vascular endothelial growth factor (VEFG), which, was previously known as vascular permeability factor is a key agent in the physiologic and pathologic angiogenesis[7]. The human VEGF family is comprised of VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor[8,9]. In addition to angiogenesis, VEGF A (most commonly studied) causes increased vascular permeability, which is responsible for the development of DME. It mediates this action through activation of vascular endothelial growth factor receptor-2 (VEGF-R2) and its permeability is 50,000 times more than histamine[10].

Table 1: Pre and post injection parameters in the treated eyes.

Parameters	Mean		Median		*P* value
	Pre-injection	Post-injection	Pre-injection	Post-injection	
Serum VEGF A (pg/ml)	347.8 SD ± 608	107.2 SD ± 107	175.98	85	0.000
Vitreous VEGF A (pg/ml)	701.9 SD ± 944	82.7 SD ± 136.9	307.1	21.23	0.000
Central macular sub field thickness (µm)	410 SD ± 158.99	299.6 SD ± 104.9	356	257	0.000
Macular cube volume (mm^3)	12 SD ± 2.4	11.4 SD ± 1.75	12.1	11	0.000
Macular cube average thickness (µm)	355.7 SD ± 67.9	304.69 SD ± 59.22	336	302	0.000

Table 2: Effect of intra-vitreal Bevacizumab on the macular OCT of the other eye.

Parameters	Mean		Median		*P* value
	Pre-injection	Post-injection	Pre-injection	Post-injection	
Central macular sub field thickness (µm)	345.42 SD± 143.95	282.8 SD ± 102.32	308	248	0.001
Macular cube volume (mm^3)	11.9 SD ± 2.61	11.2 SD ± 2.99	11.1	11.1	0.008
Macular cube average thickness (µm)	329.2 SD ± 71.86	318.1 SD ± 63.6	309	309	0.001

To combat the deleterious effects of high VEGF A levels, Bevacizumab was introduced. It is a highly specific, 149 kDa, recombinant, humanized monoclonal antibody that selectively binds to human vascular endothelial growth factor[11]. It was the first US FDA approved drug to neutralize the biologic activity of VEGF in cancer patients. The estimated half-life of Bevacizumab is approximately 20 days[12] (range 11 – 50 days). Recently, it is widely used off – label for the treatment of many retinal conditions including DME. In vitreous, its half-life is 9.8 days[13].

In the current study, serum and vitreous VEGF A levels were high in patients of DME and a direct effect of Bevacizumab was seen on central sub field thickness of the injected and the fellow non-injected eyes. The results were in concordance with the existing studies[5,14,15]. In many of our patients, levels of

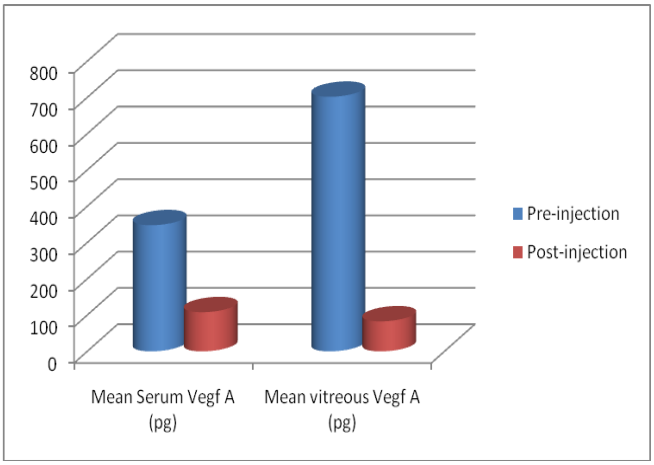

Fig. 1: Mean serum and vitreous VEGF A values before and four weeks after intra-vitreal Bevacizumab injection.

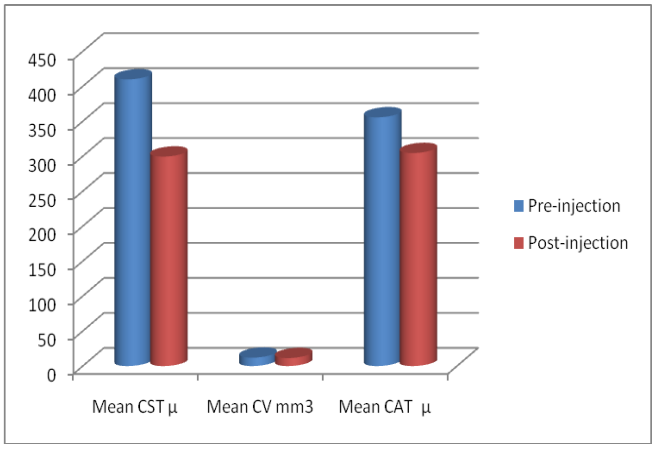

Fig. 2: Mean Central subfield thickness (CST), Mean Cube volume (CV) and Mean Cube average thickness before and after intra-vitreal Bevacizumab in the treated eyes.

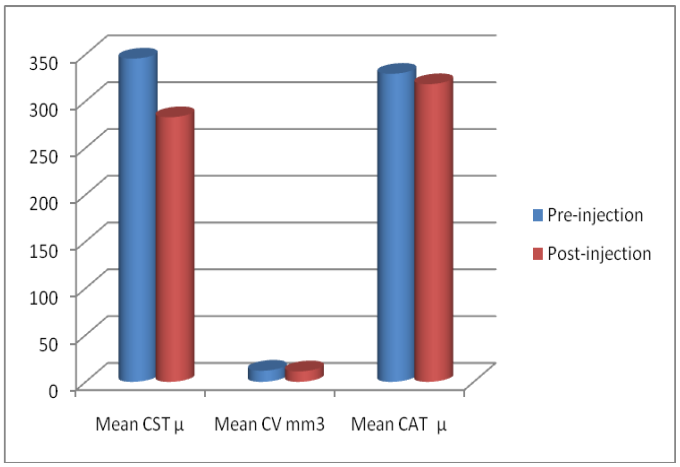

Fig. 3: Mean Central subfield thickness (CST), Mean Cube volume (CV) and Mean Cube average thickness of the other untreated eyes before and after intra-vitreal Bevacizumab in the treated eyes.

VEGF were in thousands pictograms; the highest vitreous VEGF A value was 4469.76 pg/ml and in serum the highest recorded reading was 3867.5 pg/ml in our study. These VEGF A levels in serum and vitreous were significantly reduced (p = 0.000) four weeks after intra-vitreal Bevacizumab. This indicates the leakage of Bevacizumab in the systemic circulation after intra-vitreal injection. It was similar to the previous studies in which the maximum reduction in serum VEGF A was seen on the seventh day.[16-18] in our patients, it was significantly high even after four weeks.

To further prove the leakage of intra-vitreal Bevacizumab into systemic circulation, some researchers labeled Bevacizumab with 125-I and observed radioactivity in serum[19,20]. It was noted that there was more leakage of Bevacizumab in systemic circulation in DME as compared to other diseases like neovascular Age related Macular Degeneration[21]. This can be explained by the disturbance of blood retinal barrier in diabetic retinopathy.

The Fc fragment of Bevacizumab is bound to vascular endothelium which increases the translocation across the blood-retina barrier and it also prevents elimination of Bevacizumab from systemic circulation[12,22]. This results in increased half life of Bevacizumab as compared to other anti-VEGF agents like Ranibizumab[18].

Detailed review of serum VEGF A in our study showed values distributed over a wide range. We compared them with other studies but absolute serum VEGF values were found to differ dramatically[17]. One explanation found in literature is that the platelets contain large concentrations of VEGF A. During sample collection and preservation, these platelets are broken down releasing VEGF, which is responsible for the extra ordinary large values as seen in some cases[23].

In the current study, central sub field thickness, macular volume and cube average thickness in the fellow non-injected eyes were also taken into account. The results clearly consolidated the idea of significant systemic absorption of Bevacizumab after intra-vitreal injection. This effect on the fellow non-injected eyes is also reported by other authors[24,25].

The proof of this finding was augmented by the animal studies, in which Bevacizumab injected in one eye appeared in the fellow eye after sometime[26] in contrast to this, Laser photocoagulation in one eye does not cause a decrease in the VEGF A of the fellow eyes. This shows intra-vitreal Bevacizumab has an additional benefit when compared with laser photocoagulation.

Once the systemic absorption and its effects on serum VEGF and macular thickness of other eyes is established, the question arises about the serious adverse events (SAE) associated with Bevacizumab. SEA associated with Bevacizumab are severe bleeding, including central nervous system hemorrhage, thrombo-embolic events Gastrointestinal Perforations, Surgery and Wound Healing Complications, Hypertensive Crisis, Reversible Posterior Leukoence-phalopathy Syndrome, Proteinuria and death with

systemic administration. Although in all intra-vitreal anti-VEGF registration trials, the incidence of these SAE are negligible but caution should be exercised because Bevacizumab has a cumulative effect due to its long half life[12].

There are certain shortcomings in this study. Firstly, the functional endpoint (improvement in visual acuity) was missing in this study. Visual results are dependent on many factors including status of photoreceptors and the duration of DME. We did not take into account the integrity of IS/OS layer which directly affects the visual outcome. Further elaborative studies are required to see the effect of Bevacizumab on the morphology of photoreceptor layer. Secondly, the follow up duration was short.

CONCLUSION

There is a direct relation of vitreous VEGF A, serum VEGF A and central sub field thickness. Vitreous VEGF A, serum VEGF A and central sub field thickness decrease correspondingly after intra-vitreal Bevacizumab. There is a statistically significant absorption of intra-vitreal Bevacizumab into systemic circulation. Hence, caution should be exercised in high-risk patients (Ischemic heart disease, stroke etc.). It was also concluded that the effect of intra-vitreal Bevacizumab on the fellow eyes is an additional benefit not seen with laser photocoagulation.

Acknowledgement

This study was funded by OSP Lateef Chaudhary research fund.

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